Study Number 02-S503

Report Title Abamectin

Independent Laboratory Validation of Syngenta Analytical Method 115-00 for the Determination of NOA-422601 (Abamectin B1a), NOA-421704 (Abamectin B1b), and NOA-427011 (8,9-Z Abamectin B1a) in Water

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Summary

The analytical procedure documented in Syngenta study report 115-00 (Appendix 7) was independently validated by laboratory personnel totally unfamiliar with the method and using equipment (supplies and instrumentation) not previously used by the method developer.

River water control samples were fortified at the method LOQ (0.05 µg L^{-1}) and at 0.5 µg L^{-1} in quintuplet, and analysed using the supplied analytical procedure. The recoveries of NOA-422601 (Abamectin B1a), NOA-421704 (Abamectin B1b) and NOA-427011 (8,9-Z Abamectin B1a) were determined for each sample and the results used to determine the validity of the method.

Validation was successful on the first attempt. However, the initial analysis by high performance liquid chromatography with mass spectrometric detection (LC-MS-MS) produced low recovery values for NOA-421704. After consultation with the sponsor, experiments were carried out to determine the cause of the problem and it was established that the low recovery values were caused by matrix suppression of the instrument response. The samples were re-injected using a reduced injection volume to minimise the matrix suppression and successful results were obtained.

The control samples analysed as part of this study showed no residues of NOA-422601, NOA-421704 and NOA-427011 above the limit of detection (LOD).

The response of the LC-MS-MS for NOA-422601, NOA-421704 and NOA-427011 was linear over the range tested 0.25 to 10 ng mL^{-1} (equivalent to 2.5 to 100 pg of analyte injected onto the column) with a correlation coefficient (R^2) of 1.0000, 0.9968 and 0.9999 respectively and the intercepts close to zero.

1 Introduction

The aim of this study was to achieve an independent laboratory validation of the analytical method described in Syngenta study report 115-00 (Appendix 7) for the determination of NOA-422601, NOA-421704 and NOA-427011 in water. Specifically :

a) To prove that procedural recoveries of water fortified with NOA-422601, NOA-421704 and NOA-427011 can be taken through the analytical method, and a mean recovery of 70-120% of the fortified amount can be achieved with an overall relative standard deviation (RSD) of ≤ 20%.

b) To demonstrate that the relationship between sample concentration and detector response is linear over the working range of the method.

The validation was carried out by personnel totally unfamiliar with the method and using equipment (supplies and instrumentation) not previously used by the method developer, thus meeting the requirements as permitted in OPPTS 850.7100 and EPA guidelines PR Notice 96-1.

2 Materials

2.1 Test and Reference Substances

The following analytical standards were utilised in this study.

Figure 1

Compound : NOA-422601 (Abamectin B1a)

CAS Number : 65195-55-3

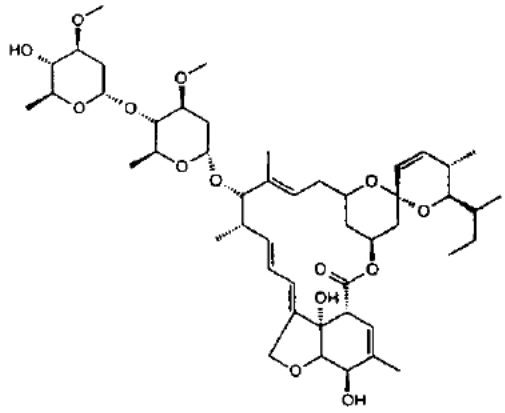


Figure 2

Compound : NOA-421704 (Abamectin B1b)

CAS Number : 65195-56-4

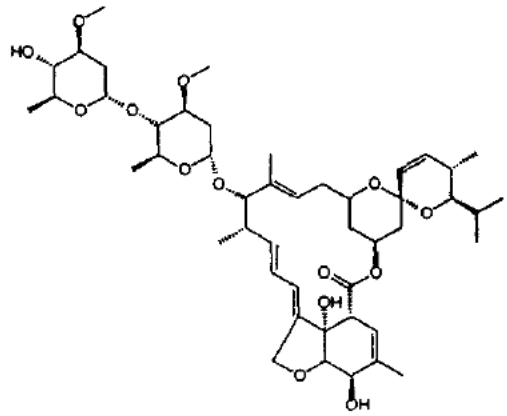
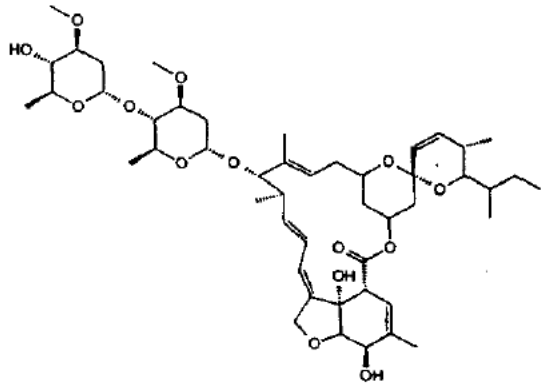


Figure 3

Compound : NOA-427011 (8,9-Z Abamectin B1a)

CAS Number : 113665-89-7



NOA-422601 Analytical Standard Reference [Batch Reference MRH-I-35-2]. Purity 99.5%. Expiry date November 2002.
NOA-421704 Analytical Standard Reference [Batch Reference SCR-I-47]. Purity 99.1%. Reanalysis date June 2004.
NOA-427011 - Analytical Standard Reference [Batch Reference TEW-IX-5-1]. Purity 98.8%. Reanalysis date December 2002.

The analytical standards were obtained from Syngenta Crop Protection, Analytical Development and Product Chemistry GS2131, Münchwilen AG, Breitenloh 5, CH-4333 Münchwilen and Syngenta Crop Protection Inc. PO Box 18300, Greensboro NC 27419, USA. These standards are stored in the Environmental Sciences standard store at <-18°C and were used within their expiry date.

2.2 Test System

The matrix utilised in this study is detailed in Table 2.

Table 2. : Water Sample Used for Independent Lab Validation

Sample Number	Source	pH	Silt Content (% w/w)	Dissolved Organic Carbon (DOC) (mg L^{-1})	Total Hardness as $CaCO_3$ (meq L^{-1})
ABA-WATILV/1	River Thames, Wargrave, Berkshire, UK	7.60	<1	<2.0	291

Data from NRM Report No. NRMR-0560 - Analysis of Water for Certain Chemical and Physical Properties

3 Methods

3.1 Preparation and Stability of Analytical Standard Solutions

Stock standard solutions of NOA-422601, NOA-421704 and NOA-427011 were independently prepared in acetonitrile, according to the procedures described in the analytical method (Appendix 7). These were subsequently used to prepare mixed working standard solutions in acetonitrile/water (50:50%, v/v).

NOA-422601, NOA-421704 and NOA-427011 in acetonitrile or acetonitrile/water (50:50%, v/v) are assumed to be stable when stored in amber bottles at ≤ 8°C for up to 4 months after preparation.

3.2 Fortification Levels

Recovery of the analytes through the analytical procedure was assessed by fortifying aliquots of river water with NOA-422601, NOA-421704 and NOA-427011. Five replicate recoveries were carried out at the LOQ (0.05 µg L^{-1}) and five replicate recoveries were carried out at ten times the LOQ (0.5 µg L^{-1}) Fortification levels are summarised in Table 3. In addition, two control samples and one reagent blank were analysed with the sample batch.

Table 3. : NOA-422601, NOA-421704 and NOA-427011 Fortification Levels

Fortification Level ($\mu g\ L^{-1}$)	Number of Replicates
Control	2
0.05	5
0.5	5

3.3 Analytical Procedures

3.3.1 Sample Analysis

Samples were analysed according to procedures described in detail in Syngenta study report 115-00 (Appendix 7). The method was followed as written, with the following modifications:

- LC-MS-MS instrument conditions – minor modifications were made to the instrument parameters in order to maximize sensitivity. (see Appendix 1 for full details of analytical instrumentation).

- HPLC mobile phase – an isocratic mobile phase of methanol/water (88:12, v/v) with an analysis time of 6 minutes was used. The mobile phase was changed in order to eliminate autosampler carry over which was seen when using the gradient program specified in method 115-00.

The percentage recovery obtained for each sample was calculated and these results were used to assess the relative standard deviation of the analytical method.

3.3.2 Detector Linearity

Standard solutions containing NOA-422601, NOA-421704 and NOA-427011 at concentration levels from 0.00025 to 0.01 $\mu g\ mL^{-1}$ (equivalent to 2.5 to 100 pg of analyte injected onto the column) in triplicate were analysed by LC-MS-MS, using the conditions specified in the analytical method and the modifications listed in Section 3.3.1. The mean detector response was plotted against standard concentration.

3.3.3 Time Required for Analysis

Based on experience gained during this study, it is estimated that a batch of up to 13 samples could be prepared for analysis within one working day, with chromatographic determination overnight (see Table 8, Appendix 2).

6 Recommendations

The following comments on the method indicate where minor improvements or clarification may be useful.

- Table 1 : HPLC System and Operating Conditions.

  Due to carry-over problems with the autosampler used for this study (CTC HTS PAL), it was necessary to modify the mobile phase from a gradient to an isocratic mobile phase of methanol/water (88:12, v/v).

Instrument Description

Pump	:	Agilent 1100 series quaternary pump model number G1311A
Degasser	:	Agilent 1100 series model number G1322A
Column Oven	:	Agilent 1100 series model number G1316A fitted with column switching valve
Detector	:	Applied Biosystems API 3000 triple quadrupole mass spectrometer
Autosampler	:	CTC HTS PAL

Chromatography Conditions

Column	:	Phenyl hexyl 150 mm × 2.0 mm, 5 µm particle size
Mobile phase	:	Methanol/ 0.2% (v/v) acetic acid in water (88:12, v/v)
Column flow rate	:	0.3 mL min^{-1}
Injection volume	:	10 µL
Stop Time	:	6 minutes
Column oven temperature	:	40°C

Mass Spectrometer Conditions – NOA-422601 and NOA-427011

Interface	:	TurboIonSpray
Polarity	:	Positive
Nebuliser gas (NEB)	:	Nitrogen set at 12 (arbitrary units)
Curtain gas (CUR)	:	Nitrogen set at 8 (arbitrary units)
Temperature (TEM)	:	400°C
Ionspray voltage	:	5500 V
Collision gas setting (CAD)	:	Nitrogen set at 8 (arbitrary units)
Scan type	:	MRM
Q1 mass	:	895.5
Q3 mass	:	751.5
Dwell time	:	300 ms

Resolution Q1	:	Low
Resolution Q3	:	Unit
Declustering potential (DP)	:	195 V
Focusing potential (FP)	:	350 V
Entrance potential (EP)	:	10 V
Collision energy (CE)	:	59 V
Collision cell exit potential (CXP)	:	26 V
Electron multiplier setting (CEM)	:	2600 V

Mass Spectrometer Conditions – NOA-421704

Interface	:	TurboIonSpray
Polarity	:	Positive
Nebuliser gas (NEB)	:	Nitrogen set at 12 (arbitrary units)
Curtain gas (CUR)	:	Nitrogen set at 8 (arbitrary units)
Temperature (TEM)	:	400°C
Ionspray voltage	:	5500 V
Collision gas setting (CAD)	:	Nitrogen set at 8 (arbitrary units)
Scan type	:	MRM
Q1 mass	:	881.5
Q3 mass	:	737.5
Dwell time	:	300 ms
Resolution Q1	:	Low
Resolution Q3	:	Unit
Declustering potential (DP)	:	195 V
Focusing potential (FP)	:	350 V
Entrance potential (EP)	:	10 V
Collision energy (CE)	:	59 V
Collision cell exit potential (CXP)	:	26 V
Electron multiplier setting (CEM)	:	2600 V